

## CLAIMS

- [1] A method of judging a biological activity in an environment contaminated with an organochlorine compound that is at least one of  
5 tetrachloroethylene (PCE) and trichloroethylene (TCE), the method comprising:
- amplifying a nucleic acid extracted from an environmental sample by a gene amplification method so as to use the nucleic acid as a target;
  - hybridizing the target to at least one DNA probe including a base  
10 sequence unique to each of at least one type of bacterium related to degradation of the organochlorine compound so that the at least one type of bacterium in the environment is detected; and
  - judging capability of the environment to eliminate the organochlorine compound based on degrading capability of the at least one type of bacterium  
15 that is detected with respect to the organochlorine compound and a dechlorinated product thereof,
  - wherein the at least one DNA probe includes a DNA probe containing any one of types of polynucleotides described below in (1) to (4), and
  - the at least one type of bacterium related to degradation of the  
20 organochlorine compound is at least one type of an anaerobic bacterium selected from a group consisting of types of bacteria denoted below as A to R:
- (1) A polynucleotide comprising any one of base sequences represented by SEQ ID NOS: 1 to 17 and SEQ ID NOS: 19 to 105 of the Sequence Listing, respectively.
  - 25 (2) A polynucleotide comprising a base sequence obtained by deletion, substitution or insertion of one to several bases in the base sequence of the polynucleotide described in (1), which is hybridizable to a polynucleotide comprising a base sequence complementary to the polynucleotide described in (1) under a stringent condition.
  - 30 (3) A polynucleotide comprising a base sequence obtained by deletion,

substitution or insertion of one to several bases in the base sequence of the polynucleotide described in (1), which has a homology of 90% or higher with the polynucleotide described in (1).

- (4) A polynucleotide comprising a base sequence complementary to any one of the polynucleotides described in (1) to (3).

A: Dehalospirillum multivorans

B: Desulfitobacterium frappieri

C: Actinomycetales Sm-1 (Rhodococcus sp. Sm-1)

D: Rhodococcus rhodococcus

10 E: Xanthobacter flavus

F: Mycobacterium L1

G: Desulfomicrobium norvegicum (Desulfovibrio baculatus)

H: Desulfitobacterium dehalogenans

I: Desulfitobacterium hafniense

15 J: Clostridium formicoaceticum

K: Desulfuromonas chloroethenica

L: Acetobacterium woodii DSM 1030

M: Dehalobacter restrictus

N: Desulfitobacterium sp. strain PCE1

20 O: Desulfitobacterium frappieri TCE1

P: Acetobacterium woodii DSM 2396

Q: Desulfomonile tiedjei DCB-1

R: Dehalococcoides ethenogenes 195.

[2] The method according to claim 1,

- 25 wherein the gene amplification method with respect to the nucleic acid uses as a sense primer, a primer that contains a polynucleotide comprising a base sequence represented by SEQ ID NO: 116 of the Sequence Listing, and as an antisense primer, a primer that contains a polynucleotide comprising a base sequence represented by SEQ ID NO: 117 of the Sequence Listing and/or a primer that contains a polynucleotide comprising a base
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sequence represented by SEQ ID NO: 118 of the Sequence Listing.

[3] The method according to claim 1,  
wherein the contaminated environment is selected from a group  
consisting of soil, groundwater, pond water and seawater.

5 [4] The method according to claim 1,  
wherein a DNA probe for detecting the bacterium A contains any one  
of the types of polynucleotides described in (1) to (4) that corresponds to any  
one of SEQ ID NOS: 1 and 19 to 25 of the Sequence Listing.

[5] The method according to claim 1,  
10 wherein a DNA probe for detecting the bacterium B contains any one  
of the types of polynucleotides described in (1) to (4) that corresponds to any  
one of SEQ ID NOS: 2 and 26 to 30 of the Sequence Listing.

[6] The method according to claim 1,  
wherein a DNA probe for detecting the bacterium C contains any one  
15 of the types of polynucleotides described in (1) to (4) that corresponds to any  
one of SEQ ID NOS: 3 and 31 to 35 of the Sequence Listing.

[7] The method according to claim 1,  
wherein a DNA probe for detecting the bacterium D contains any one  
of the types of polynucleotides described in (1) to (4) that corresponds to any  
20 one of SEQ ID NOS: 4 and 36 to 40 of the Sequence Listing.

[8] The method according to claim 1,  
wherein a DNA probe for detecting the bacterium E contains any one  
of the types of polynucleotides described in (1) to (4) that corresponds to any  
one of SEQ ID NOS: 5 and 41 to 45 of the Sequence Listing.

25 [9] The method according to claim 1,  
wherein a DNA probe for detecting the bacterium F contains any one  
of the types of polynucleotides described in (1) to (4) that corresponds to any  
one of SEQ ID NOS: 6 and 46 to 48 of the Sequence Listing.

[10] The method according to claim 1,  
30 wherein a DNA probe for detecting the bacterium G contains any one

of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 7 and 49 to 53 of the Sequence Listing.

[11] The method according to claim 1,

wherein a DNA probe for detecting the bacterium H contains any one  
5 of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 8 and 54 to 57 of the Sequence Listing.

[12] The method according to claim 1,

wherein a DNA probe for detecting the bacterium I contains any one  
of the types of polynucleotides described in (1) to (4) that corresponds to any  
10 one of SEQ ID NOS: 9 and 58 to 62 of the Sequence Listing.

[13] The method according to claim 1,

wherein a DNA probe for detecting the bacterium J contains any one  
of the types of polynucleotides described in (1) to (4) that corresponds to any  
one of SEQ ID NOS: 10 and 63 to 68 of the Sequence Listing.

15 [14] The method according to claim 1,

wherein a DNA probe for detecting the bacterium K contains any one  
of the types of polynucleotides described in (1) to (4) that corresponds to any  
one of SEQ ID NOS: 11 and 69 to 74 of the Sequence Listing.

[15] The method according to claim 1,

20 wherein a DNA probe for detecting the bacterium L contains any one  
of the types of polynucleotides described in (1) to (4) that corresponds to any  
one of SEQ ID NOS: 12 and 75 to 79 of the Sequence Listing.

[16] The method according to claim 1,

wherein a DNA probe for detecting the bacterium M contains any one  
25 of the types of polynucleotides described in (1) to (4) that corresponds to any  
one of SEQ ID NOS: 13 and 80 to 86 of the Sequence Listing.

[17] The method according to claim 1,

wherein a DNA probe for detecting the bacterium N contains any one  
of the types of polynucleotides described in (1) to (4) that corresponds to any  
30 one of SEQ ID NOS: 14 and 87 to 91 of the Sequence Listing.

[18] The method according to claim 1,  
wherein a DNA probe for detecting the bacterium O contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 15 and 92 to 96 of the Sequence Listing.

5 [19] The method according to claim 1,  
wherein a DNA probe for detecting the bacterium P contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 16 and 97 to 99 of the Sequence Listing.

[20] The method according to claim 1,  
10 wherein a DNA probe for detecting the bacterium Q contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 17 and 100 to 105 of the Sequence Listing.

[21] The method according to claim 1,  
wherein in a case where at least one of the types of bacteria denoted  
15 as J, L and P is detected, it is judged that the environment has the capability to degrade PCE into TCE.

[22] The method according to claim 1,  
wherein in a case where at least one of the types of bacteria denoted  
as A, G and M is detected, it is judged that the environment has the  
20 capability to degrade PCE into cis-dichloroethylene (cisDCE).

[23] The method according to claim 1,  
wherein in a case where at least one of the types of bacteria denoted  
as B, I, H, N, O and Q is detected, it is judged that the environment has the  
capability to degrade PCE and TCE into cisDCE.

25 [24] The method according to claim 1,  
wherein in a case where the type of bacterium denoted as K is detected, it is judged that the environment has the capability to degrade PCE and TCE into DCE.

[25] The method according to claim 1,  
30 wherein in a case where the type of bacterium denoted as R is

detected, it is judged that the environment has the capability to degrade PCE, TCE, DCE and vinyl chloride (VC) into ethene.

[26] The method according to claim 1,

wherein in a case where at least one of the types of bacteria denoted  
5 as C, D and E is detected, it is judged that the environment has the capability to degrade DCE and VC into carbon dioxide.

[27] The method according to claim 1,

wherein in a case where the type of bacterium denoted as F is  
detected, it is judged that the environment has the capability to degrade VC  
10 into carbon dioxide.

[28] A bioremediation method with respect to an environment  
contaminated with an organochlorine compound that is at least one of PCE  
and TCE, the method comprising steps of:

judging a biological activity in the environment by the method  
15 according to claim 1; and

stimulating, when a bacterium related to degradation of the  
organochlorine compound is detected, growth and/or an activity of the  
bacterium so as to enhance the degradation of the organochlorine compound  
or a dechlorinated product of the organochlorine compound.

20 [29] A bioremediation method with respect to an environment  
contaminated with an organochlorine compound that is at least one of PCE  
and TCE, the method comprising steps of:

judging a biological activity in the environment by the method  
according to claim 1; and

25 adding at least one of types of bacteria related to degradation of the  
organochlorine compound other than a detected bacterium to the  
environment so as to enhance the degradation of the organochlorine  
compound or a dechlorinated product of the organochlorine compound.

[30] A device for detecting the at least one type of bacterium used in the  
30 method according to claim 1, comprising as a DNA probe, any one of the types

of polynucleotides described in (1) to (4).

[31] The device according to claim 30,  
wherein at least two DNA probes as claimed in claim 30 are included,  
and at least two of the at least one type of bacterium can be detected at the  
5 same time.

[32] A DNA microarray that is used in the method according to claim 1,  
comprising a substrate on which a DNA probe containing any one of the types  
of polynucleotides described in (1) to (4) is immobilized.

[33] The DNA microarray according to claim 32,  
10 wherein at least two DNA probes as claimed in claim 32 are  
immobilized, and at least two of the at least one type of bacterium can be  
detected at the same time.

[34] A kit for detecting the at least one type of bacterium used in the  
method according to claim 1, comprising:  
15 a DNA probe containing any one of the types of polynucleotides  
described in (1) to (4); and  
a primer for gene amplification and a reagent for gene amplification  
that are used for preparing a target to be hybridized to the DNA probe so as  
to be detected.

20 [35] A kit for detecting the at least one type of bacterium used in the  
method according to claim 1, comprising:  
the DNA microarray according to claim 32; and  
a primer for gene amplification and a reagent for gene amplification  
that are used for preparing a target to be hybridized to the DNA probe so as  
25 to be detected.

[36] A primer that is used as a sense primer in a gene amplification  
method with respect to a nucleic acid in the method according to claim 1,  
comprising a polynucleotide comprising a base sequence represented by SEQ  
ID NO: 116 of the Sequence Listing.

30 [37] A primer that is used as an antisense primer in a gene amplification

method with respect to a nucleic acid in the method according to claim 1, comprising a polynucleotide comprising a base sequence represented by SEQ ID NO: 117 or 118 of the Sequence Listing.

[38] A DNA probe for detecting the bacterium A that can be used in the  
5 method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 1 and 19 to 25 of the Sequence Listing.

[39] A DNA probe for detecting the bacterium B that can be used in the  
10 method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 2 and 26 to 30 of the Sequence Listing.

[40] A DNA probe for detecting the bacterium C that can be used in the  
15 method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 3 and 31 to 35 of the Sequence Listing.

[41] A DNA probe for detecting the bacterium D that can be used in the  
method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 4 and 36 to 40 of the Sequence Listing.

20 [42] A DNA probe for detecting the bacterium E that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 5 and 41 to 45 of the Sequence Listing.

[43] A DNA probe for detecting the bacterium F that can be used in the  
25 method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 6 and 46 to 48 of the Sequence Listing.

[44] A DNA probe for detecting the bacterium G that can be used in the  
30 method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID



NOS: 7 and 49 to 53 of the Sequence Listing.

[45] A DNA probe for detecting the bacterium H that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID  
5 NOS: 8 and 54 to 57 of the Sequence Listing.

[46] A DNA probe for detecting the bacterium I that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID  
NOS: 9 and 58 to 62 of the Sequence Listing.

10 [47] A DNA probe for detecting the bacterium J that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID  
NOS: 10 and 63 to 68 of the Sequence Listing.

[48] A DNA probe for detecting the bacterium K that can be used in the  
15 method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID  
NOS: 11 and 69 to 74 of the Sequence Listing.

[49] A DNA probe for detecting the bacterium L that can be used in the method according to claim 1, comprising any one of the types of  
20 polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID  
NOS: 12 and 75 to 79 of the Sequence Listing.

[50] A DNA probe for detecting the bacterium M that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID  
25 NOS: 13 and 80 to 86 of the Sequence Listing.

[51] A DNA probe for detecting the bacterium N that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID  
NOS: 14 and 87 to 91 of the Sequence Listing.

30 [52] A DNA probe for detecting the bacterium O that can be used in the

method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 15 and 92 to 96 of the Sequence Listing.

5 [53] A DNA probe for detecting the bacterium P that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 16 and 97 to 99 of the Sequence Listing.

[54] A DNA probe for detecting the bacterium Q that can be used in the method according to claim 1, comprising any one of the types of  
10 polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 17 and 100 to 105 of the Sequence Listing.

[55] A polynucleotide that can be used as a DNA probe for detecting a bacterium related to degradation of an organochlorine compound in the method according to claim 1, the polynucleotide being any one of types of  
15 polynucleotides described below in (1) to (4):

(1) A polynucleotide comprising any one of base sequences represented by SEQ ID NOS: 1 to 17 and SEQ ID NOS: 19 to 105 of the Sequence Listing, respectively.

(2) A polynucleotide comprising a base sequence obtained by deletion,  
20 substitution or insertion of one to several bases in the base sequence of the polynucleotide described in (1), which is hybridizable to a polynucleotide comprising a base sequence complementary to the polynucleotide described in (1) under a stringent condition.

(3) A polynucleotide comprising a base sequence obtained by deletion,  
25 substitution or insertion of one to several bases in the base sequence of the polynucleotide described in (1), which has a homology of 90% or higher with the polynucleotide described in (1).

(4) A polynucleotide comprising a base sequence complementary to any one of the polynucleotides described in (1) to (3).